UDC 618.177-089.888.11-048.56-07-08
DOI: 10.15587/2519-4798.2024.296672

ASSESSMENT OF THE IMPLANTATION WINDOW AND EMBRYONIC FACTOR IMPACT TO THE TREATMENT OF RECURRENT IMPLANTATION FAILURE (RIF). A PROSPECTIVE STUDY

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The aim: *to study of the prognostic value of endometrial receptivity and preimplantation genetic diagnosis of embryos, and their influence on the effectiveness of in vitro fertilization (IVF) programs. We also evaluate the importance of this factor in comparison with other potential causes of infertility.*

Materials and methods: *This prospective cohort study included 123 infertile women who underwent in vitro fertilization (IVF) treatment. 93 patients had repeated unsuccessful implantation attempts and were divided into three groups: group 1 – patients who were treated using genetically untested embryos according to a standard fixed stimulation protocol, group 2 – patients who were treated using euploid embryos after preimplantation genetic screening according to standard fixed protocol; group 3 – patients who underwent treatment using euploid embryos after pre-implantation genetic screening and determination of the implantation window with subsequent modification of the stimulation protocol, according to the endometrial examination result. 30 patients had a first attempt at IVF, which was carried out using genetically untested embryos, according to a standard fixed protocol, and made up the control group (CG).*

Determination of the window of implantation was carried out by triple aspiration biopsy of the endometrium during the luteal phase of the menstrual cycle since the endometrium is most susceptible to implantation during this period. Samples were analyzed using scanning electron microscopy. Based on the results obtained, the endometrial preparation protocol was individualized for the next attempt. preimplantation genetic testing (PGT) of embryos was carried out by the next generation (NGS) method.

Statistical analysis was performed using IBM SPSS V25.0 for Windows software.

Results: *According to the obtained results, patient characteristics, screening rates, IVF cycle characteristics, and the number, quality, and stage of transferred embryos were compared between groups. The rate of clinical pregnancy was 46.7 % among patients of group 1.70 % among patients of group 2, 82.8 % among patients of group 3 and 50.0 % of the control group and statistically significantly different between groups (χ^2=10.955, p=0.012). The rate of live birth was 43.3 % among patients of group 1, 53.3 % among patients of group 2, 72.4 % among patients of group 3 and 43.3 % - of the control group, however, it did not differ statistically significantly between groups (χ^2=6.639, p=0.084)*

Conclusions: *The unique window of implantation and the embryonic factor are among the main reasons for multiple failed implantation attempts. Personalization of the endometrial preparation protocol and preimplantation embryo diagnosis are effective methods to improve IVF outcomes*

Keywords: *implantation window (IW), pipelle biopsy, implantation failure, preimplantation genetic testing (PGT), in vitro fertilization (IVF)*

How to cite:
Kozyra, O., Medvediev, M. (2024). Assessment of the implantation window and embryonic factor impact to the treatment of recurrent implantation failure (RIF). A prospective study. ScienceRise: Medical Science, 1 (58), 13–26. doi: http://doi.org/10.15587/2519-4798.2024.296672

1. Introduction

Infertility is an urgent problem of medicine and society. Socio-demographic and economic conditions contribute to a negative trend among the population of reproductive age in Europe [1]. In vitro fertilization (IVF) is the most common method of infertility treatment; however, does not guarantee pregnancy. According to the available data, about 60 % of couples who seek medical help in specialized institutions must undergo a second attempt, and some need three or more IVF procedures [2]. The embryonic factor accounts for approximately one-third of the reasons for the failure of in vitro fertilization treatment, the rest is due to implantation problems [3]. There are well-characterized morphological and molecular markers of implantation, but the complete dynamics of the process, as well as the relative importance of each step in the process, remain unclear [4].

One important factor in IVF failure is the lack of synchrony between endometrial maturation and embryo development, as this can lead to reduced endometrial receptivity and lack of implantation. The receptivity of

the endometrium is a complex process that provides the embryo with the opportunity to attach, penetrate the body and develop further, be born and continue the species [5]. The period of time when the endometrium is receptive to blastocyst implantation is called the implantation window. During this period, the plasma membrane of the endometrial epithelium loses microvilli, and a dome-shaped protrusion, called pinopods, is formed on the apical surface of the cells [6]. The formation of pinopods during the luteal phase [7] is one of the main indicators of the readiness of the endometrium for embryo implantation, and the assessment of this condition has been proposed as one of the markers of endometrial receptivity [8, 9]. The implantation window is genetically determined and occurs 6–7 days after the luteinizing hormone (LH) surge. Day 0 is the day of peak LH levels before ovulation. The formation of foam pods before or after LH + 6/7 can lead to unsuccessful implantation of the embryo in an IVF attempt since the day of embryo transfer is fixed.

One of the key factors in the treatment of infertility is the embryonic factor, the importance of which in the process of achieving a live birth of a healthy child is critical. It has been observed that blastocyst aneuploidy can significantly limit the potential to achieve this goal of treatment. Research data emphasize that the quality and genetic stability of the embryo plays a decisive role in the establishment of pregnancy and the development of a healthy foetus [10]. Preimplantation genetic testing (PGT) was first used to determine the sex of embryos in 1990. Until 2010, the study was performed using a biopsy of the embryo at the stage of cleavage by the method of fluorescence in situ hybridization (FISH); however, blastocyst biopsy (trophectoderm; TE biopsy) became mainstream in 2012. In addition, comparative genomic hybridization (aCGH) was used for analysis, which later evolved into next-generation sequencing (NGS), which is now used worldwide for euploidy screening and mutation diagnosis [11].

The study aimed to evaluate the importance of using embryo transfer personalization because of the implantation window study in combination with preimplantation genetic testing in patients with multiple failed implantation attempts.

The novelty of the study consists in evaluating the effectiveness of determining the window of implantation and pre-implantation genetic diagnosis of the embryo both in a complex and separately, determining the feasibility of using these methods at various stages of infertility treatment by the method of assisted reproductive technologies (ART). Also, the evaluation of the effectiveness of the programs in our study was carried out at the level of live births, which makes the study scientifically valuable and relevant for clinical practice [12, 13].

2. Materials and methods

The study was carried out during the period from 2020 to 2023 and included 120 women of reproductive age based on LLC "Rodyne Dzherelo". All patients previously signed an informed voluntary consent to participate in the study.

Hormone replacement therapy was used to prepare the endometrium and determine the implantation window. Oestrogen was started on day 2 or 3 of the cycle with oral oestradiol valerate at a dose of 4 mg, and this dose was increased to 6 mg per day on day 7 or 8 of the cycle. Progestins were used from day 13–15 of the cycle at a dose of 400 mg per day intravaginally after the endometrial thickness was more than 7 mm. Endometrial samples were obtained by papillary biopsy. Biopsy was performed three times during the artificial cycle on the 6th, 8th and 10th days of progestin administration.

All patients of the prospective stage of the study were examined in accordance with the order of the Ministry of Health of Ukraine No. 787 dated September 9, 2013, the local clinical protocol "Repeated implantation failures" and the recommendations of the European Society of Human Reproduction and Embryology.

The meeting of the local commission on bioethics was held by LLC "Rodyne Dzherelo" on January 4, 2022, protocol No. 1. Informed voluntary consent of patients regarding participation in the study was drawn up and approved. All patients gave written consent.

Endometrial tissues were gently washed in phosphate-buffered saline (PBS) to remove blood and surface debris and placed in fixative. A small part of each sample was fixed in 2.5 % glutaraldehyde and, after several washes in buffer, dehydrated by increasing the concentration of ethanol (25 % / 50 % / 75 %). Samples were transferred in ethanol to a Samdri 780A critical point dryer. Dried with liquid carbon dioxide, mounted on aluminium scanning electron microscopic pins, and sputtered with gold: palladium alloy (50:50) to a thickness of 300 nm using a Gatan. pecs 682 tool. Scanning electron microscopy (SEM) was performed using a Tescan Mira 3 LMU microscope. All SEM parameters, such as accelerating voltage, working distance, magnification, and field of view, are presented in the photomicrograph (Fig. 1).

Pinopods were defined as smooth apical projections from the surface epithelium without microvilli. The expression of pinopods was evaluated as follows: absence of pinopods, beginning of the formation of pinopods; formed foam pods, regression of foam pods.

Patients with an established shift of the implantation window made up the study group (group 3). A reattempt of the IVF program for the patients of this group was carried out considering the individual characteristics of the implantation window. Patients of group 1, group 2 and CG were treated by the IVF method according to a fixed protocol - embryo transfer was carried out on the 6th day of progestin use. Scanning endometrial microscopy was not performed in patients of these groups.

To optimize the statistical analysis of embryo quality assessment and its impact on the results of IVF programs, the Istanbul Consensus classification of 2011 was used [14]. The embryos of good and top-quality patients of group 2 and group 3 underwent preimplantation genetic diagnosis.

Preimplantation genetic testing (PGT) of embryos is a genetic analysis that allows you to obtain information about the number and structure of chromosomes in a human embryo before implantation in the uterine cavity. PGT is carried out with the aim of selecting embryos without genetic disorders for subsequent embryo transfer. The trophectoderm cells obtained by biopsy of embryos

at the stage of blastocyst development were subject to testing. Embryos of the appropriate size and stage of development (blastocyst III (AA AB VA BB), blastocyst IV (AA AB VA BB), blastocyst V (AA AB VA BB)) were subjected to the procedure, which were previously laser hatched on the 3rd day of cultivation. The trophectoderm cells to be harvested were captured with a biopsy needle, stretched, cut with a laser, and released from the biopsy pipette into the medium. Biopsies were subjected to tubing, freezing into marked straws. A pneumatic injector and biopsy pipettes of size XS or M, Biopsy buffer (-20 °C), PCR tubes, Petri dishes, PVS (Polyvinylpyrrolidone) +PBS (Phosphate-buffered saline), Liquid paraffin, Nikon Eclipse Ti-U microscope, Narishige Takanome micromanipulator system, Hamilton Thorn Silos-tk laser hatching system, Hamilton Thorn Silos-tk laser hatching system, Tokai Hit ThermoPlate TP-108 heating system were used for the biopsy.

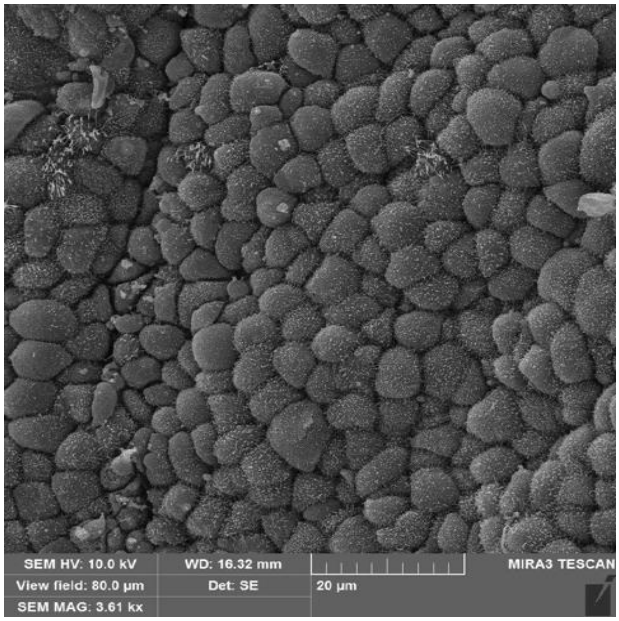

Fig. 1. Image of an electron scanning microscope. The endometrium is at the stage of foam formation

Frozen biopsies were to be transported to a genetic laboratory, where PGT was performed by the next generation sequencing (NGS) method.

The nature of the distribution of quantitative traits was assessed both by visual graphic method and by using Kolmogorov-Smirnov & Lilliefors test for normality and Shapiro-Wilk's test of normality. Since the conducted evaluation of the indicators determined significant differences from the normal nature of the distribution, the methods of non-parametric statistics were used in the calculations. Thus, to characterize the central tendency and variability of quantitative variables (continuous or interval), the median (Me) and the values of the lower (Lower quartile; QL) and upper (UQ; Upper quartile) were determined. The result was given in the form of Me [LQ; UQ]. The probability of differences in quantitative indicators in two unrelated groups was determined using the Mann-Whitney U-test. The probability of differences in quantitative indicators in two related groups was determined by the Wilcoxon signed-rank test. Qualitative (binomial, ordinal, nominal) indicators were described in absolute and relative (percentage) values. The result was given in the form of abs. values (%). The comparison of groups on a qualitative basis was carried out using the formation of four-field or arbitrary tables and the application of the Pearson's chi-squared test with the corresponding value of the $\chi 2$ criterion. Statistical analysis was performed using IBM SPSS V25.0 for Windows software.

3. Research results

This prospective cohort study included 123 infertile women undergoing in vitro fertilization (IVF) treatment. 93 patients had repeated unsuccessful implantation attempts and were divided into three groups: group 1 – patients who were treated using genetically untested embryos according to a standard fixed stimulation protocol, group 2 – patients who were treated using euploid embryos after preimplantation genetic screening according to standard fixed protocol; group 3 – patients who underwent treatment using euploid embryos after pre-implantation genetic screening and determination of the implantation window with subsequent modification of the stimulation protocol, according to the endometrial examination result. 30 patients had their first attempt at in vitro fertilization (IVF), which was carried out using genetically untested embryos, according to a standard fixed protocol, and made up the control group (CG). The average age of patients was 34.4 years, with a range from 26 to 45 years, and this indicator did not show statistically significant differences between the studied groups – 34.0 [31.8; 37.5], 34.0 [30.0; 39.3], 34.0 [31.5; 38.0] and 33.5 [30.75; 39.00] in groups 1, 2, 3 and the control, respectively (p1-2=0.629, p1-3=0.692, p1-к=0.935, p2-3=0.569, p2-к=0.588, p3-к=0.952). Primary infertility was identified as the dominant form of infertility in all groups, with a frequency of 73.3 % in group 1, 66.7 % in group 2, 82.8 % in group 3, and the highest rate of 90.0 % in the control group (χ^2=5.552, p=0.136).

During the analysis of various infertility factors between groups, it was established that statistically significant differences were found only in the case of the tubal-peritoneal factor, where the frequency of this factor was statistically significantly different between groups (Table 1).

In particular, combined infertility was observed in 90.0 % (27) of cases in group 1. A slightly lower frequency, but statistically insignificant (χ^2=0.233, p=0.342) proportion of patients in group 2 and group 3 had combined infertility – 86.7 % (26) and 82.8 % (24) of cases, respectively. The frequency of combined infertility in the control group was 73.3 % (22 cases).

As part of the study, it was established that gynecological diseases are present in a significant part of the participants of the prospective groups. However, data analysis showed the absence of statistically significant differences in the frequency of these diseases between groups: in group 1, gynecological diseases were identified in 93.3 % (28 cases) of women, in group 2 – in 83.3 % (25 cases), in group 3 – in 86.2 % (25 cases), and in the control group – in 90.0 % (27 cases) (χ^2=1.650, p=0.972). The detailed structure of gynecological morbidity is presented in the Table 2.

Table 1

Infertility factors (abs., %)

Factor	Control	Group 1	Group 2	Group 3	χ^2 p
Chronic anovulation	13 (43.3)	6 (20.0)	13 (43.3)	6 (20.7)	7.240 0.065
Age	11 (36.7)	7 (23.3)	8 (26.7)	9 (32.1)	1.493 0.684
Tubular-peritoneal	7 (23.3)	16 (53.3)	9 (30.0)	6 (20.7)	9.080 0.028
Decreased ovarian reserve	13 (43.3)	9 (30.0)	13 (43.4)	8 (27.6)	2.755 0.431
Uterine	13 (43.3)	19 (63.3)	15 (50.0)	13 (44.8)	2.969 0.396
Male	16 (53.3)	14 (46.7)	14 (46.7)	12 (41.4)	0.853 0.837
Immunological	3 (10.0)	6 (20.0)	8 (26.7)	9 (31.0)	4.368 0.224
Genetic	1 (3.3)	1 (3.3)	0 (0.0)	2 (6.9)	2.159 0.540
Endocrine	11 (36.7)	7 (23.3)	11 (36.7)	8 (27.6)	1.880 0.598
Unspecified infertility	5 (16.7)	0 (0.0)	2 (6.7)	2 (6.9)	6.064 0.109
Social	3 (10.0)	3 (10.0)	1 (3.3)	5 (18.5)	3.569 0.312

Table 2

The structure of gynecological morbidity (abs., %)

Disease	Control	Group 1	Group 2	Group 3	χ^2 p
Uterine leiomyoma	7 (23.3)	8 (26.7)	7 (23.3)	8 (27.6)	0.233 0.972
Endometriosis	9 (30.0)	16 (53.3)	9 (30.0)	11 (37.9)	4.631 0.201
Adenomyosis	7 (23.3)	12 (40.0)	8 (26.7)	10 (34.5)	2.384 0.497
Endometrioid ovarian cysts	3 (10.0)	3 (10.0)	4 (13.3)	2 (6.9)	0.675 0.879
Pelvic inflammatory diseases	7 (23.3)	16 (53.3)	9 (30.0)	6 (20.7)	9.080 0.028
Hyperplastic processes of the endometrium	1 (3.3)	3 (10.0)	7 (23.3)	3 (10.3)	6.069 0.108
Anomalies of the Muller duct	3 (10.0)	5 (16.7)	3 (10.0)	5 (17.2)	1.239 0.744
Endometrial polyp	6 (20.0)	8 (26.7)	8 (26.7)	5 (17.2)	1.155 0.764
Abnormal uterine bleeding	3 (10.0)	1 (3.3)	1 (3.3)	2 (6.9)	1.677 0.642
Sexually transmitted infections	10 (33.3)	20 (66.7)	8 (26.7)	8 (27.6)	13.602 0.004
Polycystic ovary syndrome	6 (20.0)	5 (16.7)	9 (30.0)	5 (17.2)	2.069 0.558
Benign diseases of the mammary gland	7 (23.3)	6 (20.0)	5 (16.7)	5 (17.2)	0.535 0.911
Asherman syndrome	1 (3.3)	0 (0.0)	0 (0.0)	1 (3.4)	2.070 0.558
Ovarian cysts	6 (20.0)	5 (16.7)	6 (20.0)	2 (6.9)	2.517 0.472

In the structure of gynaecological morbidity, the most significant share was accounted for by endometriosis. Thus, adenomyosis was detected in almost a third of patients in all prospective groups: 40.0 % (12) – group 1,

26.7 % (8) – group 2, 34.5 % (10) – group 3, 23.3 % (7) - control group. There was no statistically significant difference between the groups (χ^2=2.384, p=0.497). Of note is the frequency of sexually transmitted infections in group 1 (16 (53.3 %)) compared to group 2, group 3 and the control group (9 (30.0 %), 6 (20.7 %), 7 (23.3 %) respectively). The difference is statically reliable (χ^2=9.080 p=0.028), correlated with the frequency of inflammatory diseases of the pelvic organs. The least common were Asherman's syndrome, abnormal uterine bleeding, and anomalies of the Mullerian duct.

Hormonal homeostasis was evaluated by determining the following hormonal indicators: follicle-stimulating hormone (FSH), luteinizing hormone (LH), prolactin (PRL), and anti-Müllerian hormone (AMH) (Table 3).

A characteristic feature of the concentration of these hormones was wide fluctuations in its values in the groups. Thus, the level of FSH fluctuated between 1.14–110.5 mIU/ml. A statistically significant difference in the level of FSH was observed between group 3 and the control group (6.73 mIU/ml [4.17, 9.73], 4.37 mIU/ml [2.97, 6.55], p=0.011, respectively). Decreased levels of FSH were noted in 23.3 % of cases among women of group 1, 26.7 % – among patients of group 2, 37.9 % – among patients of group 3, 13.3 % – among patients of the control group and did not statistically differ between groups (χ^2=8.221, p=0.222). Instead, the frequency of elevated FSH levels varied significantly between groups - 10.0 %, 20.0 %, 3.4 % and 16.7 % of patients in groups 1, 2, 3 and the control group, respectively (χ^2=8.221, p=0.222).

A similar trend was observed in the case of LH concentration. The hormone concentration ranged from 0.1 to 73.6 mIU/ml. A statistically significant difference in LH level was observed between group 3 and the control group (6.16 mIU/ml [4.12, 9.03], 3.20 mIU/ml [1.22, 6.30], p=0.006, respectively), and between group 2 and group 3 (6.14 mIU/ml [3.48; 11.13], 3.20 mIU/ml [1.22; 6.30], p=0.006, respectively). Decreased levels of LH were found in 23.2 % of cases among women of group 1 and 10.0 % of cases of patients of group 2, 44.8 % of cases of patients of group 3 and 6.7 % of the control group; instead, they were elevated in 6.7 %, 13.3 %, 0.0 %, and 13.3 % of cases, respectively, and were statistically significantly different between groups (χ^2=18.368 p=0.005).

Levels of AMH concentration were comparable - no statistically significant difference was established between the values in the prospective groups.

The level of prolactin concentration ranged from 3.81 to 110.0 ng/ml and was significantly different between group 2 (11.28 ng/ml [7.71; 16.78]) and group 3 (16.90 [11.33; 27.16] p=0.041). However, the level of prolactin after the treatment of patients with an elevated level of the hormone at the screening stage did not have a statistically significant difference between the groups. The frequency of increased prolactin levels during the screening examination was 16.7 % in group 1 and the control group, 13.3 % in group 2, 28.6 % in group 3 (χ^2=6.264 p=0.394). All patients were re-examined with determination of molecular forms and therapy with cabergoline drugs was carried out.

To evaluate the function of the thyroid gland, the following indicators were determined: thyroid-stimulating hormone (TSH), the level of antibodies to thyroid peroxidase (ATPO), as well as antibodies to thyroglobulin (ATTG) (Table 4).

Table 3

Indicators of hormones, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	pK-1	pK-2	pK-3	p1-2	p1-3	p2-3
FSH, mIU/ml	6.73 [4.17; 9.73]	5.11 [3.52; 7.38]	4.91 [3.11; 11.28]	4.37 [2.97; 6.55]	0.800	0.344	0.011	0.693	0.437	0.306
LH, mIU/ml	6.16 [4.12; 9.03]	5.51 [2.57; 8.80]	6.14 [3.48; 11.13]	3.20 [1.22; 6.30]	0.302	0.965	0.006	0.272	0.095	0.006
AMH, ng/ml	1.60 [0.57; 4.15]	2.05 [1.07; 3.69]	2.69 [0.72; 4.90]	2.21 [0.98; 3.94]	0.433	0.469	0.510	0.888	0.958	0.649
PRL, ng/ml	13.14 [10.98; 21.59]	11.87 [8.85; 16.80]	11.28 [7.71; 16.78]	16.90 [11.33; 27.16]	0.167	0.060	0.450	0.473	0.111	0.041
PRL, ng/ml control	22.75 [12.41; 27.01]	24.64 [14.78; 30.84]	11.20 [8.69; 19.86]	15.25 [11.83; 19.74]	0.461	0.114	0.170	0.148	0.172	0.203

Table 4

The value of the concentration of indicators of thyroid gland function, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	pK-1	pK-2	pK-3	p1-2	p1-3	p2-3
TSH, mIU/l	2.05 [1.38; 3.08]	1. 90 [1.14; 2.59]	1.72 [1.21; 2.57]	1.80 [1.23; 3.33]	0.539	0.214	0.820	0.641	0.510	0.367
TSH mIU/l control	3.07 [2.44; 3.36]	3.03 [2.57; 3.38]	1.55 [1.33; 2.22]	2.09 [1.51; 2.55]	0.864	0.018	0.003	0.032	0.012	0.276
ATPO, IU/ml	3.13 [0.50; 12.20]	1.95 [0.48; 11.53]	8.80 [3.40; 18.52]	11.00 [3.45; 18.25]	0.704	0.069	0.064	0.024	0.020	0.826
ATTG, IU/ml	5.30 [0.22; 18.05]	9.35 [0.30; 38.00]	11.75 [10.00; 30.19]	32.55 [11.75; 61.25]	0.554	0.013	0.001	0.099	0.010	0.074
Euthyrox, mg	37.5 [25.0; 50.0]	50.0 [31.25; 50.0]	50.0 [31.25; 50.0]	25.0 [25.0; 50.0]	0.686	0.686	0.762	1.000	0.352	0.221

The level of the concentration of thyroid-stimulating hormone showed the opposite trends to the level of prolactin - no statistically significant difference was observed between the groups during the screening examination, however, differences in the level of TSH were recorded after the implementation of hormone replacement therapy. A statistically significant difference was established between groups 1 and 2 (3.03 mIU/L [2.57; 3.38], 1.55 mIU/L [1.33; 2.22], p=0.032), groups 1 and 3 (3.03 mIU/l [2.57; 3.38], 2.09 mIU/l [1.51; 2.55] p=0.012), control group and 2 (3.07 mIU/l [2.44; 3.36], 1.55 mIU/l [1.33; 2.22], p=0.018), control and group 3 (3.07 mIU/l [2.44; 3.36], 2.09 mIU/l [1.51, 2.55], p=0.003). The frequency of increased TSH levels did not differ between groups and amounted to 36.7 %, 26.7 %, 41.4 % and 33.3 % in groups 1, 2, 3 and the control group, respectively (χ^2=1.503, p=0, 6820). All patients were re-examined and prescribed hormone replacement therapy. The proportion of patients who used the drug L-thyroxine did not differ between groups and ranged from 13.3 % in group 1, 2 to the control group, and 20.7 % in group 3 (χ^2=0.925, p=0.820). The dosage of the drug did not differ between the prospective groups.

The ATPO and ATTG indicators were statistically significantly different between the groups, but the average values corresponded to the reference values. Elevated levels of ATPO were found in one in five patients with repeated failures and in one in seven controls. An identical trend was observed with an increase in the level of ATTG among patients with RIF, however, in the control group, the frequency of deviation was significantly lower – 3.3 % (χ^2=5.340, p=0.149).

To identify and correct the symptoms of antiphospholipid syndrome in patients with repeated unsuccessful attempts at embryo implantation (participants of groups 1, 2 and 3), a complex screening was performed.

The study included analysis of the following indicators: antibodies to cardiolipin, antibodies to phosphatidylserine, antibodies to phosphatidylethanolamine, lupus anticoagulant, and antibodies to beta-2-glycoprotein. Details of this study and its results are presented in Table 5.

Table 5

APLS screening rates, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	p1–2	p1–3	p2–3
Antibodies to cardiolipin, units/ml	–	3.13 [1.12; 6.71]	7.65 [4.43; 11.1]	4.45 [2.02; 8.33]	0.011	0.191	0.113
Antibodies to phosphatidylserine units/ml	–	5.30 [1.24; 6.98]	5.80 [2.66; 6.87]	4.26 [3.08; 6.15]	0.654	0.966	0.469
Antibodies to phosphatidylethanolamine, units/ml	–	1.35 [0.44; 3.96]	3.30 [1.12; 7.20]	6.35 [1.71; 9.28]	0.254	0.039	0.323
Lupus anticoagulant, c. u.	–	0.82 [0.67; 1.02]	1.17 [0.80; 1.36]	0.91 [0.65; 1.19]	0.086	0.636	0.219
Antibodies to beta-2-glycoprotein, units/ml	–	7.17 [2.18; 15.4]	6.20 [1.40; 11.78]	11.16 [3.11; 16.10]	0.525	0.675	0.180

The values of the above indicators by group corresponded to the reference values. All patients with an increased risk of APLS were treated with direct-acting anticoagulants (enoxaparin).

Determination of the level of homocysteine and natural killers (NK) in the blood plasma was carried out in patients of groups 1, 2 and 3. The concentration of homocysteine did not differ statistically significantly between the groups and was within the reference limits - 5.14 μmol/l [4.46; 9.66] in group 1; 5.65 μmol/l [4.56; 9.36] in group 2; 4.56 μmol/l [3.31; 9.21] in group 3, respectively (p1-2=0.496, p1-3=0.462, p2-3=0.141).

There were no differences in serum NK levels and cytotoxicity between the studied groups. The average

values correspond to the reference values – $0.24*10^9$ cells/l [0.07; 0.40], $0.14*10^9$ cells/l [0.11; 0.40], $0.13*10^9$ cells/l [0.09; 0.21] in groups 1, 2, 3, respectively (p1-2=0.350, p1-3=0.386, p2-3=0.386). An increase in the level of natural killers in blood serum was observed in 20.7 % of patients in group 1 and group 2, 25.9 % in group 3 (χ^2=7.944, p=7.944). A decrease in the level at that time was found in 20 % of patients of group 1, 13.8 % of patients of group 2 and 7.4 % of patients of group 3 (χ^2=7.944, p=7.944).

All patients with elevated levels of NK were treated with human immunoglobulins as part of the protocol of preparation for embryo transfer (Table 6).

Table 6

Indicators of immunological examination, blood serum, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	p1–2	p1–3	p2–3
NK, $*10^9$ cells/l	–	0.24 [0.07; 0.40]	0.14 [0.11; 0.40]	0.13 [0.09; 0.21]	0.350	0.386	0.386
Cytotoxicity in the ratio 10 PBMC / 1 K562 10/1, % N10-30	–	13.4 [11.0; 27.5]	15.0 [11.0; 37.3]	15.5 [11.0; 34.75]	0.720	0.959	0.959
Cytotoxicity in the ratio 10 PBMC / 1 K562 10/1, % N10-30	–	29.0 [19.0; 48.5]	27.5 [18.5; 51.5]	28.5 [21.5; 44.8]	0.867	0.986	0.986
Cytotoxicity in the ratio 10 PBMC / 1 K562 10/1, % N10-30	–	32.0 [22.0; 37.5]	24.0 [16.0; 35.5]	34.3 [17.0; 43.0]	0.302	0.396	0.396
Cytotoxicity in the ratio 10 PBMC / 1 K562 10/1, % N10-30	–	51.0 [36.0; 56.5]	34.4 [24.5; 53.0]	45.0 [33.6; 55.75]	0.116	0.231	0.231

In order to examine the endometrium, all patients with multiple negative implantation attempts underwent hysteroscopy (58.6 % – group 1, 53.6 % – group 2, 31.0 % – group 3) or aspiration biopsy (41.4 % – group 1, 46.7 % – group 2, 69.0 % – group 3). Control group patients were partially examined: hysteroscopy – 30 %, aspiration biopsy – 10 %. The frequency of examinations was statistically significantly different between the studied groups and the control group – hysteroscopy (χ^2=7.944, p=0.047) and aspiration biopsy (χ^2=19.752, p=0.001), respectively.

Data on the frequency and structure of the detected pathology in the section of the research groups are given in the Table 7.

An immunohistochemical study of the endometrium was performed to determine markers of chronic endometritis (CD 138), NK cells (CD 56), estrogen and progesterone receptors. Data on the detection of the above-mentioned in endometrial samples are given in Tables. 8–9.

The number of positively stained NK cells in the endometrial sample did not statistically significantly differ between the groups and amounted to 105.0 [44.3; 158.3] in group 1, 86.0 [38.0; –] in group 2, 147.0 [32.0; 159.5] in group 3 and 128.5 [87.0; –] in the control group (pK-1=0.533, pK-2=0.400, pK-3=0.571, p1-2=1.000, p1-3=1.000, p2-3=1.000). It is worth noting that the frequency of immunohistochemical detection of chronic endometritis is higher than the pathohistological one, while the frequency of increased NK cells in the endometrial sample is lower than in blood plasma. All patients with IHC confirmed diagnosis of chronic endometritis underwent a course of antibiotic therapy with control of treatment results.

Table 7

The structure of endometrial pathology, (abs., %)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
Identified pathology	9 (75.0)	18 (62.1)	16 (53.3)	15 (51.7)	02.358 0.502
Endometrial glandular polyp	2 (16.7)	0 (0.0)	2 (6.7)	4 (13.8)	5.007 0.171
Glandular-fibrous endometrial polyp	4 (33.3)	9 (32.1)	6 (20.0)	1 (3.4)	8.810 0.032
Foci of endometriosis	0 (0.0)	4 (14.3)	1 (3.3)	3 (10.7)	3.643 0.303
Simple endometrial hyperplasia	2 (16.7)	3 (10.7)	3 (10.0)	1 (3.4)	2.070 0.558
Hypoplastic endometrium	0 (0.0)	1 (3.6)	1 (3.3)	3 (10.3)	2.646 0.450
Chronic endometritis	3 (25.0)	7 (25.0)	6 (20.0)	10 (34.5)	1.652 0.648

Table 8

Frequency of detection of IHC markers, (abs., %)

IHC marker	Control	Group 1	Group 2	Group 3	χ^2 p
CD 138	4 (33.3)	7 (25.0)	7 (23.3)	11 (37.9)	1.903 0.593
CD 56	2 (16.7)	4 (14.3)	3 (10.0)	4 (13.8)	0.433 0.933

Table 9

Endometrial receptors for oestrogens and progestins, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	pK-1	pK-2	pK-3	p1-2	p1-3	p2-3
Oestrogen receptor alpha, %	78.0 [78.0; 78.0]	83.5 [77.5; 93.0]	83.0 [65.5; 92.0]	89.0 [78.0; 98.8]	0.588	0.783	0.560	0.455	0.436	0.186
Progesterone receptor, %	63.0 [63.0; 63.0]	90.0 [83.5; 98.0]	92.0 [84.0; 97.0]	89.0 [76.0; 95.0]	0.118	0.087	0.167	0.804	0.329	0.124

The partners of female patients of all prospective groups did not statistically significantly differ in age at the time of fertilization, the index of active sperm in the ejaculate, and the percentage of normal morphology of spermatozoones in the screening spermiogram (WHO10) (Table 10).

When evaluating the spermiogram, about half of the partners of patients with repeated unsuccessful implantation attempts were found to have normospermia. The rest of the examinees had deviations, but their frequency did not statistically differ between groups (Table 11).

Table 10

Age and quantitative indicators of the partner's spermiogram, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	pK-1	pK-2	pK-3	p1-2	p1-3	p2-3
Age of partner/SD	35.5 [32.0; 40.8]	36.0 [32.0; 39.0]	36.0 [27.0; 39.5]	35.0 [29.5; 40.0]	0.906	0.358	0.592	0.470	0.699	0.803
Concentration of spermatozoones (million/ml)	16.0 [6.5; 35.2]	17.4 [1.61; 27.1]	16.0 [8.36; 22.4]	15.6 [6.85; 23.6]	0.586	0.623	0.301	0.870	0.485	0.591
% norms morphology	3.65 [2.0; 4.0]	3.0 [1.5; 4.5]	4.0 [2.3; 4.5]	4.0 [1.5; 5.4]	0.938	0.525	0.641	0.449	0.515	0.974

Table 11

The structure of spermiogram characteristics in partners, (abs., %)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
Normospermia	8 (28.6)	11 (40.7)	13 (44.8)	17 (58.6)	5.344 0.148
Oligospermia	13 (46.4)	8 (29.6)	5 (17.2)	9 (31.0)	5.709 0.127
Asthenospermia	15 (53.6)	13 (48.1)	13 (44.8)	8 (27.6)	4.404 0.221
Teratospermia	15 (55.6)	15 (55.6)	14 (48.3)	12 (42.9)	4.589 0.204
Hypospermia	4 (14.3)	2 (7.4)	2 (6.9)	0 (0.0)	4.426 0.219
Hyperspermia	2 (7.1)	2 (7.4)	1 (3.4)	0 (0.0)	2.465 0.482
Aspermia	2 (7.1)	2 (7.4)	1 (3.4)	1 (3.4)	0.823 0.844
Cryptospermia	2 (7.1)	0 (0.0)	1 (3.4)	1 (3.4)	2.057 0.561

The MAR test was positive in every tenth patient of the prospective groups (χ^2=0.302, p=0.960). Deviations in the partner's karyotype were sporadic – 2 cases among patients of group 3 and 1 case among patients of the control group (χ^2=3.834, p=0.280). None of the partners of group 1 and group 2 patients had abnormalities in karyotyping results. Sperm extraction using TESA was applicable for every fifteenth partner of the RIF groups (χ^2=2.132, p=0.545).

Intrauterine insemination was performed in 30.0 %, 30.0 %, 48.3 % and 33.3 % of patients of groups 1, 2, 3 and controls, respectively (χ^2=2.927, p=0.403). Of them, with donor sperm – 3.3 %, 3.3 %, 17.2 % and 6.9 % of patients of groups 1, 2, 3 and controls, respectively (χ^2=5.379, p=0.145). The average number of injections was 2.0 [2.0; 5.0], 1.0 [1.0; 4.0], 3.0 [2.0; 5.0] and 2.5 [2.0; 4.25] in groups 1, 2, 3 and controls, respectively, and did not differ statistically significantly (p1-2=0.136, p1-3=0.477, p1-K=0.905, p2-3=0.072, p2-K=0.211, p3 -K= 0.472). The average number of IVF cycles was identical among groups of patients with multiple failed IVF attempts and was 2.0 [2.0; 3.0], 2.0 [2.0; 3.0] and 2.0 [2.0; 3.5] in groups 1, 2 and 3, respectively (p1-2=0.711, p1-3=0.114, p2-3=0.425).

For controlled ovarian stimulation (COS) in the prospective groups, the protocol with gonadotropin-releasing hormone (GnRH) antagonists was most often used: in 76.7 % of cases in group 1, in 70.0 % of cases in group 2, 75.9 % in group 3, 72.4 % – in the control group (χ^2=1.770, p=0.940) (Table 12).

Table 12

Distribution of COS protocol types, (abs., %)

Type of ovarian stimulation protocol	Control	Group 1	Group 2	Group 3	χ^2 p
COS protocol with GnRH antagonists	21 (72.4)	23 (76.7)	21 (70.0)	22 (75.9)	1.770 0.940
COS protocol with GnRH agonists	2 (6.9)	3 (10.0)	2 (6.7)	3 (10.3)	
Oocyte donation programs	6 (20.7)	4 (13.3)	7 (23.3)	4 (13.8)	

The average age of patients at the time of puncture was 31.0 years [29.0; 34.0], 30.0 years [25.8; 34.0], 34.0 years [30.0; 37.0] and 32.0 years [28.0; 36.3] in groups 1,2,3 and control, respectively (p1-2=0.629, p1-3=0.054, p1-4=0.591, p2-3=0.089, p2-4=0.194, p3-4=0.010), and was statistically significantly different between groups 2 and 4.

When assessing the structure of the types of applied endometrial preparation protocols, the artificial protocol (ARP), the artificial protocol with GnRH agonist suppression became dominant. Embryo transfers in superovulation stimulation protocols were isolated in control group and group 1 patients and could not be applied to group 2 and 3 patients according to the study design (Table 13).

The average age at the time of embryo transfer was 34.0 years [31.8; 37.5], 34.0 years [30.0; 39.3], 34.0 years [31.5; 38.0] and 33.5 years [30.75; 39.0] in groups 1, 2, 3 and control, respectively (p1-2=0.629, p1-3=0.692, p1-K=0.9350, p2-3=0.569, p2-K=0.588, p3-K =0.952) and statistically significantly did not differ between groups. An identical trend was observed regarding the parameters of progesterone concentration in blood serum on the day of embryo transfer and endometrial thickness in the sagittal section of the uterus, which was measured on the day of progestin support (Table 14).

The displacement of the implantation window was determined exclusively in patients of group 3. The optimal day of embryo implantation for patients of group 3 according to the results of scanning electron microscopy is 8.0 [8.0; 8.5]. In patients of groups 1, 2 and the control group, embryo transfer took place on the sixth day of luteal phase support. It is worth noting that the earliest implantation window was opened on the 4th day of progesterone administration, the latest on the 10th day.

Data on the frequency of use of additional medications and treatment methods in the embryo transfer cycle are shown in Table 15.

Table 13

The structure of applied types of endometrial preparation protocols, (abs., %)

Type of endometrial preparation protocol	Control	Group 1	Group 2	Group 3	χ^2 p
Artificial protocol (ARP)	8 (26.7)	14 (46.7)	11 (36.7)	11 (37.9)	14.007 0.525
Artificial protocol with super agonists	12 (40.0)	11 (36.7)	15 (50.0)	14 (48.3)	
Natural modified	3 (10.0)	2 (6.7)	2 (6.7)	2 (6.9)	
Natural	2 (6.7)	0 (0.0)	2 (6.7)	2 (6.9)	
COS protocol with GnRH agonists	1 (3.3)	1 (3.3)	0 (0.0)	0 (0.0)	
COS protocol with GnRH antagonists	4 (13.3)	2 (6.7)	0 (0.0)	0 (0.0)	

Table 14

Prognostic levels of progesterone and endometrial thickness in the cycle of endometrial preparation for embryo transfer, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2a	Group 2b	pK-1	pK-2	pK-3	p1-2	p1-3	p2-3
Progesterone level before transfer (I), ng/ml	24.2 [18.45; 32.1]	26.3 [18.98; 31.96]	27.36 [20.32; 30.62]	28.1 [22.65; 31.3]	0.737	0.350	0.171	0.676	0.376	0.477
Endometrial thickness before the start of progesterone (I)	8.5 [7.95; 9.50]	8.8 [8.3; 9.4]	9.05 [8.43; 9.63]	9.1 [8.1; 10.1]	0.347	0.074	0.370	0.325	0.903	0.533

Table 15

The structure of applied additional medications and treatment methods (abs., %)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
Infusion of human normal immunoglobulin, 10 %	2 (6.7)	7 (23.3)	6 (20.0)	3 (10.3)	4.318 0.229
Administration of human recombinant granulocytic colony-stimulating factor	4 (13.3)	3 (10.0)	2 (6.7)	2 (6.9)	
PRP therapy of the endometrium	1 (3.3)	1 (3.3)	0 (0.0)	0 (0.0)	2.000 0.572
Use of low molecular weight heparins	5 (16.7)	4 (13.3)	15 (10.0)	3 (10.3)	**17.393 0.001**

The average dosage of preparations of human normal immunoglobulin, 10 % was 200.0 ml [150.0; 200.0], 200.0 ml [187.5; 212.5], 200.0 ml [150.0; –] and 175.0 ml [150; –] in groups 1, 2, 3 and control, respectively, did not differ statistically significantly between groups (p1-2=0.667, p1-3=0.429, p1-4=0.800, p2-3=0.534, p2-4=0.886, p3-4=0.548).

IVF with donor oocytes was performed in every 6 patients of the prospective groups without a statistically significant difference between them - 20.0 % of patients in group 1, 23.3 % in group 2, 13.8 % in group 3, and 23.3 % of patients in the control group (χ^2=1.106, p=0.776).

The average values of transferred embryos between prospective groups showed a statistically significant difference between groups 1 and 3, 1 and 4, 2 and 3, 2 and 4 – 2.0 [1.0; 2.0] – group 1, 1.0 [1.0; 1.25] – group 2 and 1.0 [1.0; 1.0] - group 3, 2.0 [1.0; 2.0] – control group (p1-2=0.804, p1-3=0.002, p1-4=0.001, p2-3=0.004, p2-4=0.002, p3-4=0.808). The distribution of cycles in terms of the number of transferred embryos is shown in Table 16.

All transferred embryos were cultured in the laboratory up to 5–6 days of development inclusive. The distribution of cycles in relation to the age of the transferred embryos is given in the Table 17.

Table 16

The structure of applied additional medications and treatment methods, (abs., %)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
1 embryo	11 (36.7)	12 (40.0)	23 (76.7)	24 (79.3)	19.856 0.003
2 embryos	18 (60.0)	17 (56.7)	7 (23.3)	6 (20.7)	
3 embryos	1 (3.3)	1 (3.3)	0 (0.0)	0 (0.0)	

Table 17

The structure of the distribution of cycles in relation to the age of transferred embryos, (abs., %)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
Embryos of the 5th day of cultivation	22 (75.9)	22 (73.3)	24 (80.0)	22 (75.9)	0.379
Embryos of the 6th day of cultivation	7 (24.1)	8 (26.7)	6 (20.0)	7 (24.1)	0.945

Pre-implantation genetic testing of embryos was performed in all patients of groups 2 and 3. Only euploid embryos were subject to subsequent transfer into the uterine cavity. Group 1 and control group patients were not previously tested for euploidy of embryos. To optimize the statistical analysis of embryo quality assessment and its impact on the results of IVF programs, the classification of the Istanbul Consensus, 2011 was used. The distribution

varied widely within and between groups, but no statistically significant difference was found (Table 18).

The distribution of the results of IVF cycles is presented in the Tables 19 and 20.

Table 18

Distribution of the quality of transferred embryos in terms of quality, (abs., %)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
Embryo quality 1 (standardization)					
good	18 (60.0)	16 (53.3)	22 (73.3)	26 (89.7)	11.592 0.072
moderate	10 (33.3)	13 (43.3)	7 (23.3)	3 (10.3)	
bad	2 (6.7)	1 (3.3)	1 (3.3)	0 (0.0)	
Embryo quality 2 (standardization)					
good	9 (50.0)	5 (26.3)	4 (57.1)	5 (83.3)	8.921 0.178
moderate	6 (33.3)	8 (42.1)	3 (42.9)	1 (16.7)	
bad	3 (16.7)	6 (31.6)	0 (0.0)	0 (0.0)	

Table 19

Distribution of IVF results in prospective groups (%, abs.)

Indicator	Control	Group 1	Group 2	Group 3	χ^2 p
Pregnancy:					
– Biochemical	63.3 (19)	50.0 (15)	73.3 (22)	93.1 (27)	13.810 0.003
– Clinical	50.0 (15)	46.7 (14)	70.0 (21)	82.8 (24)	10.955 0.012
– Lost (frozen, miscarriage)	16.7 (5)	6.7 (2)	20.0 (6)	13.8 (4)	2.367 0.500
Number of foetuses:					
– Singleton pregnancy	84.6 (11)	91.7 (11)	87.5 (14)	77.3 (17)	2.668 0.849
– Twin pregnancy	15.4 (2)	8.3 (1)	12.5 (2)	18.2 (4)	
– Pregnancy with three fetuses	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)	
Birth resolution:					
– Per vias naturalis	46.2 (6)	58.3 (7)	50.0 (8)	42.9 (9)	0.775 0.855
– Operative	53.8 (7)	53.8 (5)	50.0 (8)	57.1 (12)	
Traditional pregnancy	43.3 (13)	43.3 (13)	53.3 (16)	72.4 (21)	6.639 0.084
Newborns, term					
Newborns, sex:					0.402 0.940
– Girls	53.3 (8)	58.3 (7)	47.1 (8)	47.1 (8)	
– Boys	46.7 (7)	41.7 (5)	52.9 (9)	52.9 (9)	
Complications during pregnancy and childbirth	46.7 (14)	46.7 (14)	60.0 (18)	72.4 (21)	5.491 0.139

Table 20

Distribution of IVF results in prospective groups, Me [LQ; UQ]

Indicator	Control	Group 1	Group 2	Group 3	pK-1	pK-2	pK-3	p1-2	p1-3	p2-3
Week of gestation	38.0 [37.5; 39.5]	38.0 [37.0; 39.8]	38.0 [37.0; 40.0]	37.0 [35.8; 38.5]	0.769	0.746	0.029	0.945	0.096	0.063
Growth, see	49.0 [48.0; 50.0]	51.0 [49.25; 54.0]	50.75 [47.0; 53.0]	47.75 [45.0; 49.25]	0.16	0.244	0.211	0.391	0.002	0.047
Weight, g	3200.0 [2940.0; 3600.0]	3585.0 [2625.0; 3897.0]	3375.0 [2500.0; 3750.0]	2707.0 [2343.75; 3521.25]	0.581	0.901	0.040	0.491	0.053	0.126

4. Discussion of research results

Early reproductive losses are a common complication in pregnancy, especially in in vivo fertilization, where they often remain an unsolved phenomenon. It is known that approximately 70 % of embryos stop their development at the stage that precedes the achievement of viability. At the same time, in more than 50 % of cases, pregnancy ends due to implantation failure. The success of implantation directly depends on the exact synchronization between the development of the embryo and the state of the endometrium. The window of implantation is defined as a short period of time when the endometrium reaches maximum receptivity, which is limited by the stages of its refractoriness. The receptivity and selectivity of the endometrium are key to the possibility of implantation of an embryo that has the potential for further development. The main causes of failed implantation, as shown in our study, are embryo aneuploidy and/or disorders in the selectivity and receptivity of the endometrium. This is supported by an increase in clinical pregnancy rates in the recurrent implantation failure (RIF) group using genetically tested embryos compared to the RIF group without prior aneuploidy screening (46.7 % vs. 70.0 %, χ^2= 10.955, p=0.012). An even greater increase in this indicator was observed in the RIF group, where an additional determination of the implantation window was performed with subsequent personalization of the endometrial preparation protocol (82.8 %). Live birth results supported the null hypothesis of a positive effect of preimplantation genetic testing (PGT) of embryos and personalization of the implantation window (43.3 %, 53.3 % and 72.4 % in groups 1, 2 and 3, respectively), although the statistical significance of this difference was not established (χ^2=6.639, p=0.084). The effectiveness of the methodology for determining the window of implantation can also be seen in the rate of early pregnancy loss, which was 13.8 % among patients of group 3 compared to the frequency of 20.0 % in group 2 and 16.7 % in the control group (χ^2=2.367, p= 0.500).

The success of IVF treatment depends on many other factors: age, hormonal background, state of the endometrium and uterus, extragenital diseases, embryonic factors such as fertilization, the rate of cleavage of the embryo, its euploidy, factors related to the partner, genetic disorders, and external factors such as both the productivity of the laboratory and the clinic, legal restrictions, and increasingly the social factor comes to the fore. All groups were homogeneous regarding the above characteristics with rare exceptions, such as the frequency of detection of sexually transmitted infections, pelvic inflammatory disease and, as a consequence, the frequency of tuboperitoneal factor infertility, which were statistically significantly higher among patients in group 1, and as well as the quality of transferred embryos. This showing varied widely between groups, however, without statistical significance.

This study focused on endometrial receptivity as a factor that can be determined and compensated for according to the literature and our null hypothesis. Modern methods of diagnosing endometrial receptivity include the following markers: endometrial thickness [15], endometrial volume [16, 17], endometrial receptivity [18], markers that can be assessed using endometrial aspiration biopsy: urocortin, activin A, decidual membrane human, protein (hDP) and interleukin-18 [19], cytokines, glycodelin, isoforms of leucine-rich alpha2-glycoprotein, LIF and TNF, interleukin-1β, TNF-α, interferon-gamma-induced protein 10, and monocyte chemoattractant protein [20], markers are assessed using hysteroscopy [21]. But, despite the large number of proposed methods, today there is no single universal and generally accepted method of assessing the ability of the endometrium to ensure embryo implantation.

The main limitation of the conducted research, which was devoted to the receptivity of the endometrium, was the effect of full-scale military operations on the territory of Ukraine on the collection of information. This significantly limited the patient samples for the study. This limitation of data collection may have affected the results and conclusions of the study, as the representativeness and size of the samples were reduced.

Prospects for further research. In the long term, innovative diagnostic systems developed based on artificial intelligence can have a significant impact on improving the effectiveness of the treatment of recurrent cases of failed implantation (RIF). These systems may include algorithms to optimize the creation and selection of embryos, thereby increasing the chances of successful implantation. For example, the use of neural networks will allow analyzing the complex characteristics of the embryo and the physiological parameters of the mother, providing more accurate selection and prediction of implantation success [22, 23].

In addition, the development of newer treatments, such as robotic surrogate mothers, may open new opportunities for RIF patients. These technologies can provide alternative ways to carry an embryo, particularly in cases where traditional treatment methods are ineffective or impossible due to medical contraindications [24].

Thus, the integration of advanced technologies based on artificial intelligence and robotics into the practice of infertility treatment has the potential not only to increase the chances of successful implantation, but also to significantly expand the possibilities for the treatment of various forms of infertility, particularly in cases where traditional methods are ineffective.

Further prospective studies are needed, including the study of genetically determined factors of endometrial receptivity underlying the initiation of IW and the management of infertility in women with repeated unsuccessful implantation attempts.

5. Conclusions

The results of the study demonstrated the effectiveness of individualized embryo transfer in a group of women with repeated negative implantation attempts. A decrease in the anthropometric parameters of live-born children, such as height and weight, is observed and associated with the individualization of embryo transfer, directly correlated with the gestational age at the time of delivery.

Preimplantation genetic testing of embryos increases clinical pregnancy and live birth rates in women with multiple failed implantation attempts.

A unique implantation window and an aneuploid embryo are some of the causes of implantation failure. Screening for IW and embryo euploidy is important for patients with repeated failed implantation attempts when other causes have been ruled out. Personalization of the endometrial preparation protocol is a method to improve IVF outcomes. Further prospective studies are needed, including the study of genetically determined factors of endometrial receptivity underlying the initiation of IW and the treatment of infertility in women with repeated unsuccessful implantation attempts.

Conflict of interests. The authors declare that they have no conflict of interest in relation to this study, including financial, personal, authorship, or any other, that could affect the study and its results presented in this article.

Funding.

The study was conducted without financial support.

Data availability

Data will be provided upon reasonable request.

Use of artificial intelligence technologies

The authors confirm that they did not use artificial intelligence technologies when creating the presented work.

Acknowledgments

We are grateful for the technical support of LLC "NanoMedTech" and Mykola Skoryk, head of the electron microscopy laboratory.

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Received date 17.01.2024
Accepted date 22.02.2024
Published date 29.02.2024

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